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INFLUENCE OF SOIL CHEMICAL AND PHYSICAL PROPERTIES ON OCCURRENCE OF *TRICHODERMA* SPP. IN EMBU, KENYA

[INFLUENCIA DE LAS PROPIEDADES QUIMICAS Y FISICAS DEL SUELO SOBRE LA OCURRENCIA DE *TRICHODERMA* SPP. EN EMBU, KENIA]

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SUMMARY

Soil samples were collected from eight land use types in Embu to assess the effects of land use on soil chemical and physical parameters and their impact on occurrence of *Trichoderma* spp. The fungus was recovered from the soil using the dilution plate and soil washing technique while soil samples were analysed for pH, total nitrogen (N), phosphorus (P), potassium (K), sodium (Na), calcium (Ca) and magnesium (Mg). Land use type (LUT), plant cover, and soil physical and chemical properties influenced *Trichoderma* occurrence. The frequency of isolation of *Trichoderma* spp. was highest in soils under napier (*Pennisetum purpureum*) followed by indigenous forests. Carbon, N, Mg and Fe were high in soils collected from forests thus influencing fungal diversity. The forests had clay loam soils with higher porosity and water retention capacity compared with the cultivated LUTs which had clay texture and high bulk density. Soils from the napier and cultivated plots had low Mn and Cu levels which may have resulted from leaching and nutrient transport. This implies land use and plant type were major determining factors for the high population of *Trichoderma* recorded in napier LUT. The diversity of soil factors observed in the fallow plots explained the influence of land management on soil physical and chemical characteristics which in turn determined the fungal distribution. The age of the fallows and usage varied from farmer to farmer causing the variations observed. Soil depth (0-20cm) did not influence soil factors though fungal diversity, abundance and evenness varied with depth suggesting the influence of other drivers.The plough/top layer of a soil profile is high in organic matter including litter and has potential to support high fungal abundance and sometimes diversity. Occurrence of *Trichoderma* spp. and distribution in soil is determined by a number of interacting biotic and abiotic factors.

Key words: Soil physical and chemical properties; *Trichoderma* spp.; land use.

INTRODUCTION

Trichoderma is a genus of fungi that is present in all soils, where they are the most prevalent culturable fungi. They are saprophytes with high antagonistic activities against soil-borne pathogens. Many species in this genus can be characterized as opportunistic avirulent plant symbionts. These abilities make them quite competitive in the soil rhizosphere and thus the fungus is available commercially for control of soil born pathogens (Butt *et al*., 2001). As more strains are being suggested for bio-control preparations, their effectiveness requires an improved understanding of their soil and root ecology.

Land conversion and intensification alter the biotic interactions and patterns of resource availability. The fertilizers for example change the soil chemical and physical properties which in turn affect the environment of soil inhabitants (Kaiser, *et al*., 1992). The chemical properties that may be altered by soil amendments include the soil fertility and pH. The physical properties of soil that are improved by amendments include soil structure, porosity, and water-holding capacity. Poor soil physical characteristics directly constrain root growth and in turn may have direct effect on microbial rhizosphere inhabitants. Several species of *Trichoderma* have been found to have antagonistic and root colonizing behavior (Subhendu and Sitansu, 2007). They are also the main decomposer of cellulose in acid soils as under acidic conditions, bacteria and actinomycetes become inactive. In other soils *Aspergillus* and *Fusarium* are known to participate more in cellulose decomposition (Saria *et al.*, 2005). Understanding the effect of soil factors on *Trichoderma* spp may provide clues to which abiotic soil factor have the most influence on the bio-control activity of the fungus. The interactions of these factors may provide information to bio-control mechanisms and their regulation in situ for use in selection of strains to be applied at different soil environments.

The objective of this study was to compare soil properties in different land use systems and their effects on the distribution of *Trichoderma* spp.

MATERIAL AND METHODS

Soil sample collection

Soil samples were collected from sixty sampling points in Embu. The sixty points, 200m apart, were systematically marked in a grid-mesh construction using GPS markings. In total the points fell within eight land use types (LUTs); Tea farming (*Camellia sinensis*), Coffee farming (*Coffea arabica*), Maize based farming (*Zea mays*), Fallow land (mainly *Digitaria abyssinica, Pennisetum cladestinum*), Napier farms (*Pennisetum purpureum*), Planted forests of Meru oak (*Vitex keniensis*), Planted forests of mixed eucalyptus (*Eucalyptus saligna, E. globulus*), and Indigenous forests.

Circles of 3m and 6m radius were marked round each sampling point where four and eight soil samples were taken at 0-10cm and 10-20cm depths using soil auger respectively. The samples were mixed thoroughly to make a composite sample which was used for *Trichoderma* isolation The soil were from the sixty points were placed in paper bags then transported to the laboratory where they were stored at 2- 5^{o}C.

Isolation of *Trichoderma* spp.

Trichoderma spp. were isolated from the 120 soil samples using the soil dilution plate (Johnson *et al*., 1959) and soil washing methods (Gams *et al*., 1987; Bills & Polishook, 1994). The dilution plate method was used for the estimation of the fungus. $^{1}/_{10}$, $^{1}/_{100}$, $^{1}/_{1000}$ dilutions of the samples were prepared. Before the setting of the organic matter and soil particles, one milliliter of the dilutions were applied to plates containing malt extract (MEA) and cornmeal agar (CMD) with 2% dextrose) both with streptomycin 50mg/L and cyclosporin 10mg/L antibiotics.

For isolation using the soil washing technique, 10g of soil was sieved in a nest of 4.0 mm, 1.0 and 0.5 mm sieve. This was done by suspending 10g of the soil in two litres tap water and pouring through the nest of the sieves. The procedure was then repeated with 2L of sterile water. After this treatment, the contents of the first mesh which were large organic particles were surface sterilized by transferring the contents into a sterile Petri dish with sterile water containing streptomycin. Organic particles floating on the surface of the water and the washed soil particles were picked up with a loop and forceps and transferred onto plates of MEA and CMD (Cornmeal agar with 2% dextrose) both with streptomycin 50mg /L and Cyclosporine 10mg/L antibiotics. Two replicates per media were used. The small pieces of debris retained on the other two sieves could not be surface sterilized because they were too small and porous. The debris was damp-dried on sterile paper towels and then dried over silica gel for 24 hours before plating on the isolation media. The plates were incubated at 25^{o}C for two weeks (Gams *et al*., 1987).

The colonies were counted and identified using the soil dilution plate method. The identified colonies were transferred to Petri dishes containing PDA (potato dextrose agar) and incubated at 15, 25, 30 and 35^{o}C for further identification to species level. Colonies developed from the isolates using the soil washing technique were also identified.

Identification of *Trichoderma* species

Genus identification of green fungus was carried out using the method of Domsch *et al*., (1980). *Trichoderma* isolates were identified at species level following the taxonomic key of the genus *Trichoderma* by Samuels *et al*, 2004. Colony characteristics, growth rates in culture and morphological characters were used for identification. Microscopic examination was carried out by mounting the culture in lactophenol cotton blue but for size measurements KOH and water was used as the mounting fluid. A small amount of material was placed in a drop of 3% KOH on a slide and then replaced with water.

Soil chemical characteristics

The remaining soil samples were used for pH was determined in 1:1 (w/v) soil – water suspension with pH meter. Total nitrogen was determined by the Kjeldahl method (Page *et al.,* 1982). Available nutrients (P, K, Na, Ca and Mg) were determined using Mehlich method (Anderson and Ingram, 1993).Total organic carbon was determined by oxidation, (Nelson and Sommer, 1982).

Statistical Analysis

Analysis of the distribution of *Trichoderma* across land use systems in Embu and Taita regions was done using R version 2.1.1, (Kindt and Coe, 2005). Further, ordination methods were also used to visualise species compositions in relation to the LUTs and soil physical and chemical characteristics Analysis of Variance (ANOVA) using Genstat 9th edition) was used to study sources of variations in the data.

RESULTS

From 60 soil samples 299 *Trichoderma* isolates were obtained through soil dilution and soil washing methods. These isolates were assigned to nine species, Table 1. The most frequently occurring species was *T. harziunum* which was isolated from all LUTs. Total frequency of isolation was highest in soils under napier grass LUT followed by the indigenous forest soils.

The fungus was more rich, abundant, and at 10-20cm soil depth than in surface soil at 10cm, Table 1.The abundance and diversity however, was not high at 10-20cm depth as shown by the crossing species rank abundance curve in Figure 2 and the crossing Renyi profiles plot in Fig 3a. However the fungus was more evenly distributed in depth1 than in depth 2, Figure 3b.

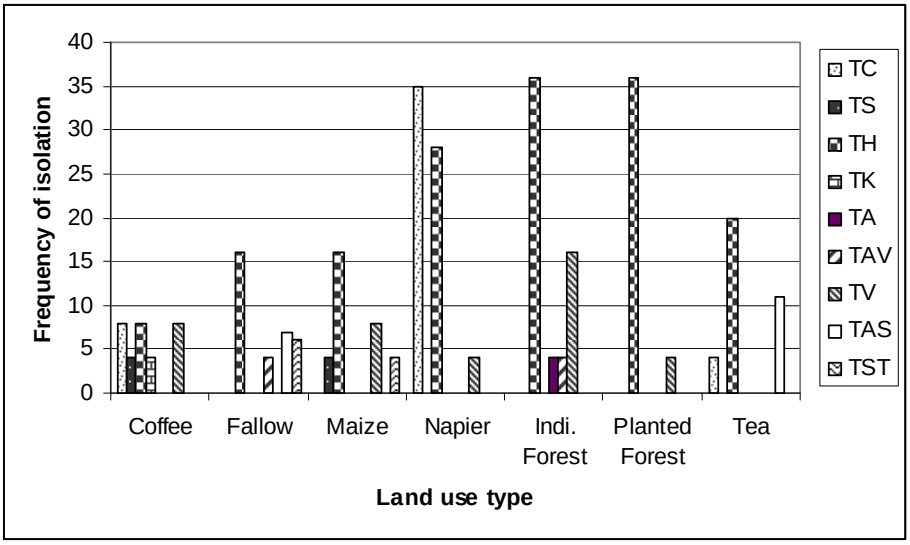


Figure 1. Frequency of isolation of *Trichoderma* species from the different land use types.
Fungal Species: TC = *Trichoderma citrinoviride,* TK = *T. koningii,* TR = *T. reesei,* TAV = *T. atroviride,* TS = *T. surrotunda,* TV = *T. viride,* TH = *T. harzianum,* TST = *T. stromaticum,* TA = *T. aggressivum,* TAS = *T. asperellum,* TP = *T. polysporum.*

Table 1: Effect of soil depth on *Trichoderma* richness, abundance and diversity

Richness

Depth	Mean	Total	Chao	Bootstrap	Jackknife
1	0.356	5	5.750	5.862	6.966102
2	0.932	8	8.444	8.998	9.966102

Abundance

Depth	Mean	Total	Jackknife
1	2629.831	155160	307690.2
2	5196.780	306610	608023.2

Shannon

Depth	Mean	Total	Jackknife
1	0.015	0.899	0.9978233
2	0.108	1.071	1.1338488

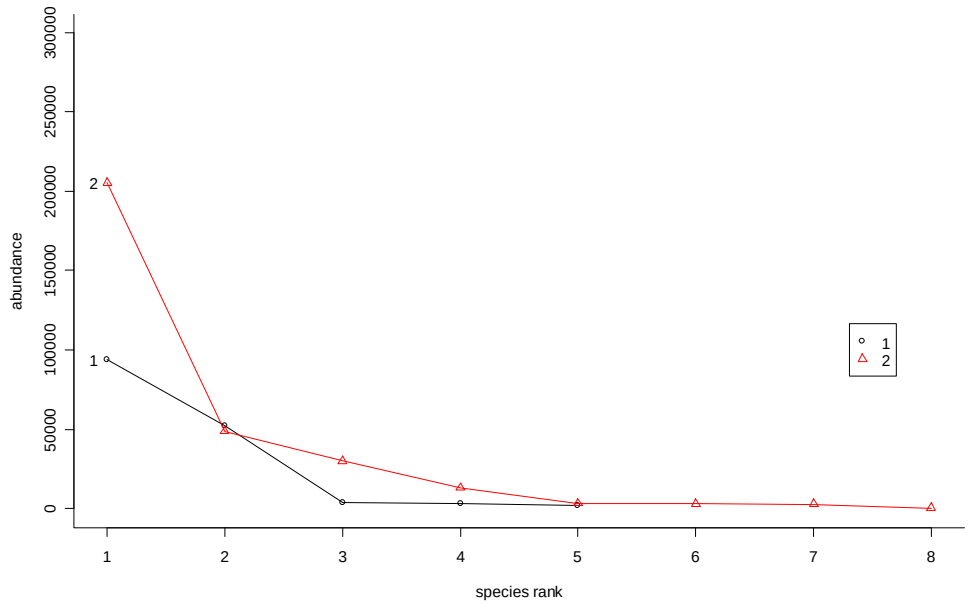


Figure 2. Rank abundance curve showing variation of *Trichoderma* abundance with depth. (Depth 1 = 0-10cm, Depth 2 = 10-20cm).

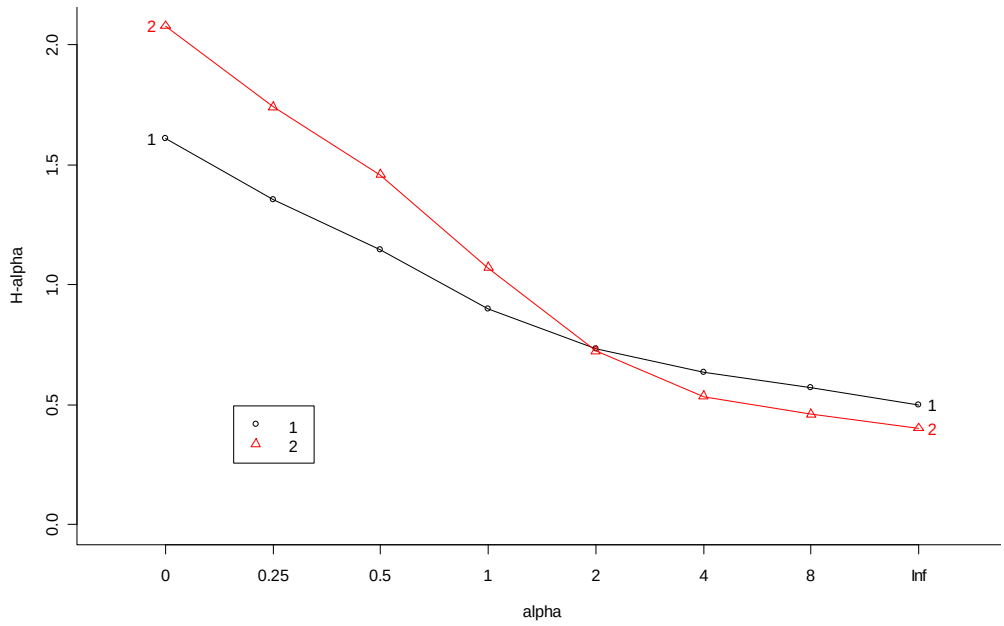


Figure 3a. Renyi profiles curve for the diversity of *Trichoderma* in soil (Depth 1 = 0-10cm, Depth 2 = 10-20cm).

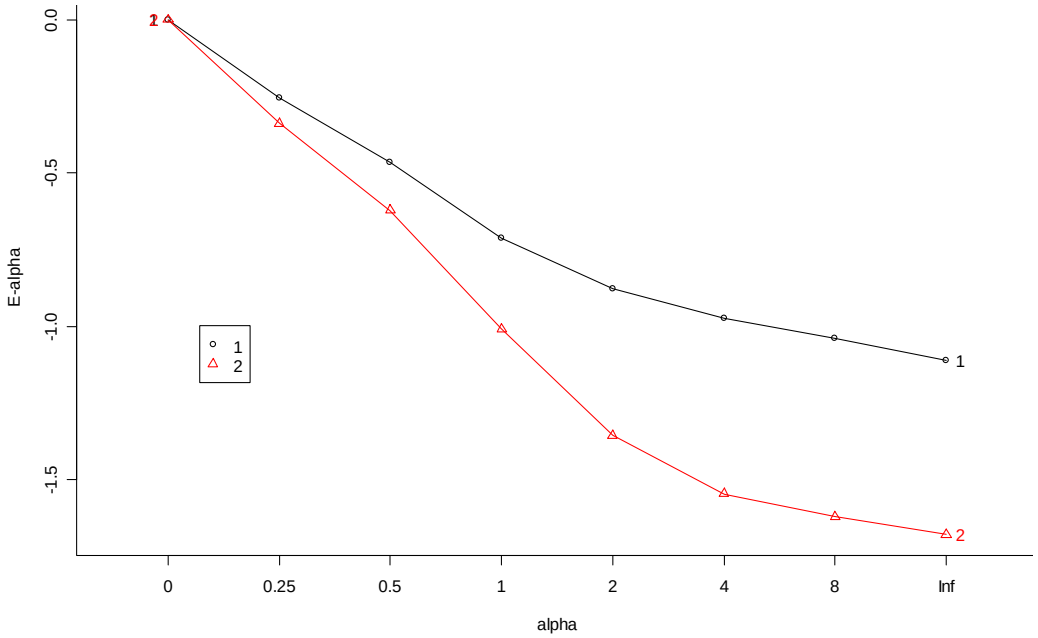


Figure 3b. Renyi profiles curve for evenness of *Trichoderma* in soil (Depth 1 = 0-10cm, Depth 2 = 10-20cm).

Chemical and physical soil properties varied significantly with land use but only K was influenced by depth, Table 2 and 3.The amount of clay was low in the forests compared to other LUTs while silt and sand was high. Available water and soil porosity was also higher in the forests and fallow LUTs compared to the LUTs and soil water retention was high in tea and fallow LUTs. Acidity, C, N, Fe, and organic matter (OM) was high in the forests and fallow LUTs. Copper was significantly high in soils under coffee while Fe was high in indigenous forests, and zinc in fallow plots. Fallow, napier and maize recorded higher pH values compared to the other LUTs.

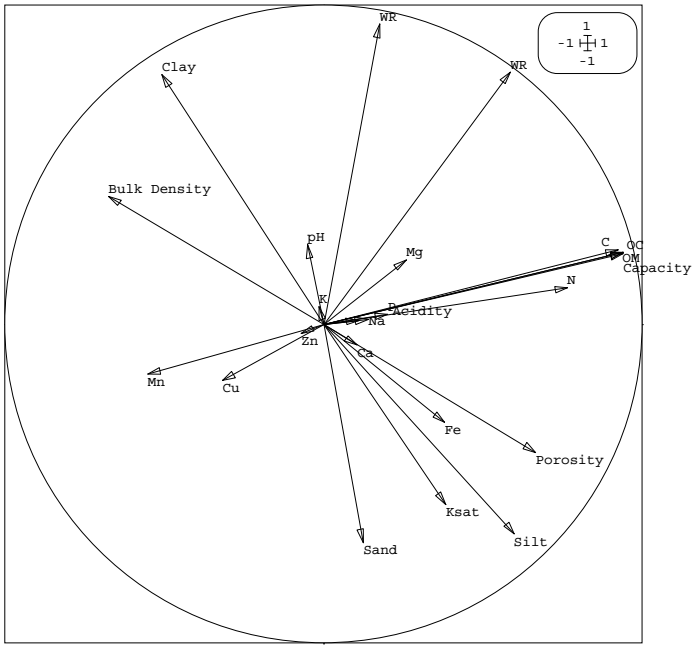
The soil properties were ordinated with LUTs and two groupings were observed; that of forests and another of cultivated land. The forests loaded with C, N, P, OM, K, Mg, Fe, water availability and retention, porosity, silt and sand, while the cultivated fields loaded with Mn, Cu, bulk density and clay. Fallow fields showed a large ellipse whose borders touched the two groups indicating variability of soil properties. The natural forest and napier showed the least variability. The first two components accounted for 45.67% of the total variations in the data as shown by the sum of eigen values for the first two factors that are used to draw the correlation graph, Fig 4. The soil texture in the forests is clay loam while that in the cultivated land is clay (Figure 4b).

Table 2. Soil physical characteristics as influenced by land use intensification.

Land Use Type	Soil physical properties					
	Clay (%)	Silt (%)	Sand (%)	Available water capacity (mm/m)	Porosity (%)	Bulk density (g/cm2)
Coffee	53.50	21.17	25.22	0.2701	64.98	0.8406
Tea	58.30	19.20	22.50	0.2847	68.27	0.7615
Maize	51.81	20.75	27.44	0.2606	63.15	0.8844
Napier	53.81	22.44	23.75	0.2685	64.48	0.8525
Fallow	51.75	24.38	23.88	0.3058	68.26	0.7619
Planted Forest	38.83	33.44	27.61	0.3217	72.78	0.6533
Indigenous Forest	39.31	33.00	27.69	0.3061	76.30	0.5687
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 3: Soil chemical characteristics as influenced by land use intensification

LUT	pH	Acidity	C	N	OM	Ca	P	Na	K	Mg	Mn	Fe	Zn	Cu
Coffee	3.841	2.049	3.78	0.337	6.50	1.81	13.00	0.236	0.379	0.577	0.542	39.1	8.38	22.58
Tea	3.534	2.865	4.51	0.381	7.75	1.89	20.95	0.142	0.244	0.527	0.392	52.1	3.45	0.62
Maize	4.205	1.069	3.32	0.371	5.82	2.38	16.19	0.345	0.464	1.019	0.666	41.0	10.34	3.89
Napier	4.209	0.906	3.59	0.319	6.17	2.28	12.62	0.228	0.230	0.733	0.691	32.7	8.82	4.41
Fallow	4.299	1.209	5.59	0.665	9.62	2.39	14.31	0.292	0.453	1.230	0.580	32.4	16.58	0.96
Planted Forest	3.928	2.094	6.39	0.806	7.75	1.98	20.95	0.142	0.211	1.264	0.166	58.8	5.11	3.57
Indigenous Forest	3.482	2.288	5.56	0.627	9.56	3.34	21.06	0.336	0.348	0.252	0.473	89.4	5.96	0.94
P-value ≤	0.001	0.001	0.001	0.001	0.001	0.048	0.003	0.001	0.001	0.031	0.001	0.001	0.001	0.001



Num.	Eigenval.	R.Iner.	R.Sum	Num.	Eigenval.	R.Iner.	R.Sum
01	+6.8361E+00	+0.2848	+0.2848	02	+4.1241E+00	+0.1718	+0.4567
03	+2.9634E+00	+0.1235	+0.5801	04	+1.8900E+00	+0.0788	+0.6589

Figure 4a. Correlation graph of the soil characterization in Embu

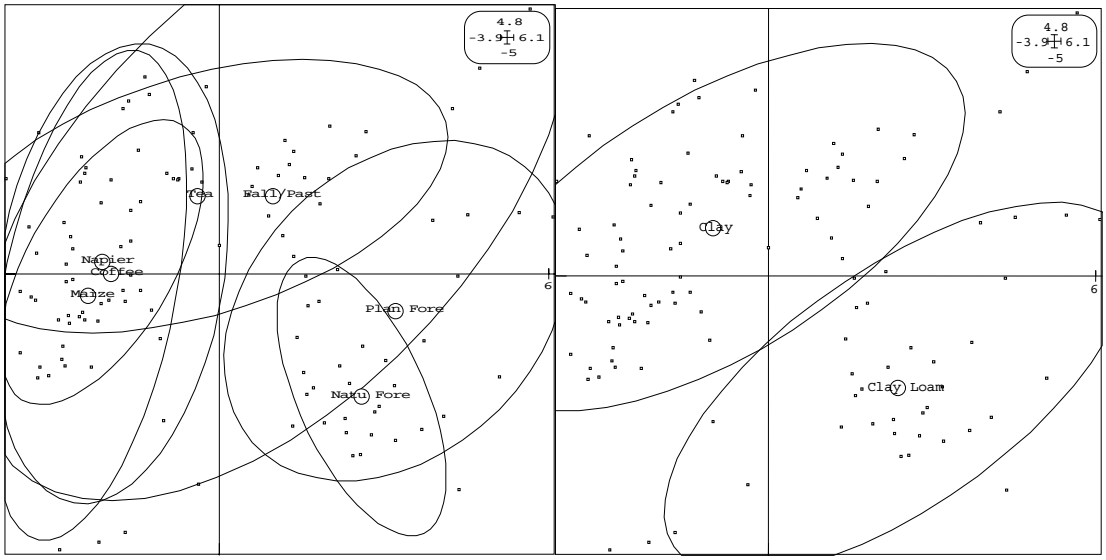


Figure 4b. Ordination Ellipses for the land use types and soil texture in relation to soil properties.

Interactions were observed among soil properties where N, Mg, Mn, and Zn were positively correlated with soil pH while acidity and Fe were negatively correlated with pH. Acidity was negatively correlated with K, Mg, Mn, and Na while Fe was positively correlated. Nitrogen was positively correlated with C, Mg and Fe but negatively correlated with Mn. Table 4 shows interactions among the soil properties with positive and negative correlation coefficients with probability values beneath them. Significant p-values at alpha=0.05 are in parentheses.

No relationship was observed between *Trichoderma* spp species and soil nutrients which implied that other factors influenced the distribution of the fungus (Figure 5). The first two factors in the ordination graph below explained 83.40% of the variance and could be substrate availability and type as observed in the results on distribution of the fungus as influenced by land cover above.

DISCUSSION

The results showed that there were many factors that interacted to determine the occurrence of *Trichoderma* spp. The most common species is *T. harziunum*. The two depths examined (0-10 and 10-20cm) did not influence the occurrence of the fungus, rather, the substrate availability in the two depths seemed to be the determining factor. The fungus was more rich and evenly distributed in the top soil but more abundant in the second depth. The diversity was not determined by depth. This suggests the presence of a favourable factor on the top soil and a different favourable one in the second depth. Studies on colonization and succession of fungi during decomposition has shown that *Trichoderma* are late colonizers of fresh litter and normally found underneath the litter on top soil, Takashi, 2005; Takashi, *et al*., 2006 where they probably degrade non-lignified holocellulose. The cellulolytic activities of the fungus explain its occurrence mostly on the top soil. The abundance of the fungus in the lower soil depth could be associated with the extensive rooting system of the napier grass which recorded the highest frequency of isolation. . Plant roots growing in soils are a major source of carbon and energy to microorganisms in the form of root exudates, cells detached from old parts of the roots or the roots itself after plant death. *Trichoderma* spp have been reported to be more rhizosphere competent than most soil fungi (Tsahouridou and Thanassoulopoulos, 2002). Younger roots produce more of these exudates than older once (, Garland, 1996, Harman and Kubicek Vol.2 1998, Picard *et al.,* 2000). This could explain why the fungus was abundant and unevenly distributed in the second depth where it would flourish in the rhizosphere with younger roots. Fungal abundance was however not high in the lower depth for all the species encountered confirming further that some *Trichoderma* species are more rhizosphere competent than others (Anderson and Gray, 1990; Harman, and Kubicek, 1998,; Subhendu and Sitansu, 2007).

Table 4. Correlation coefficients among soil properties.

Correlation matrix (Pearson):

Variables	Acidity	N	C	P	K	Ca	Mg	Mn	Cu	Fe	Zn	Na
pH	-0.540	0.188	0.038	-0.173	0.147	-0.080	0.537	0.213	-0.121	-0.369	0.285	0.151
P values	(< 0.0001)	(0.041)	0.682	0.060	0.113	0.389	(< 0.0001)	(0.020)	0.194	(< 0.0001)	(0.002)	0.103
Acidity		-0.099	0.050	0.147	-0.284	-0.145	-0.478	-0.412	-0.010	0.313	-0.180	-0.294
P values		0.287	0.591	0.113	(0.002)	0.117	(< 0.0001)	(< 0.0001)	0.917	0.051	0.879	(0.001)
Nitrogen			0.697	0.057	0.063	0.028	0.491	-0.446	-0.185	0.114	-0.014	0.188
P values			(< 0.0001)	0.540	0.500	0.761	(< 0.0001)	(< 0.0001)	(0.045)	0.217	0.806	0.204
Carbon				0.171	-0.012	0.037	0.280	-0.465	-0.299	0.223	-0.023	0.118
P values				0.064	0.898	0.693	(0.002)	(< 0.0001)	(0.001)	(0.015)	0.806	0.204
Phosphorus					-0.027	-0.043	-0.084	-0.070	-0.162	0.051	-0.058	0.001
P values					0.770	0.646	0.367	0.453	0.080	0.581	0.536	0.060
Potassium						0.215	0.190	0.254	0.147	0.063	0.073	0.333
P values						(0.019)	(0.039)	(0.005)	0.113	0.498	0.431	(0.000)
Calcium							0.006	0.039	-0.115	0.126	0.073	0.659
P values							0.952	0.679	0.214	0.175	0.435	0.389
Magnesium								-0.024	-0.079	-0.272	0.101	0.208
P values								0.798	0.395	(0.003)	0.277	(0.024)
Manganese									0.111	-0.261	0.361	0.043
P values									0.231	(0.004)	(< 0.0001)	0.642
Copper										-0.087	0.101	-0.031
P values										0.346	0.275	0.737
Iron											-0.162	0.067
P values												0.204
Zinc												0.069
P values												0.204

Positive and negative correlation coefficients with probability values beneath them. Significant p-values in parentheses. Significance level of alpha=0.05

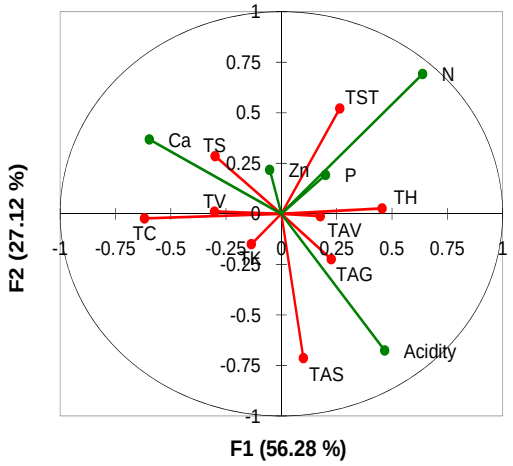


Figure 5. Correlation of *Trichoderma* occurrence with soil chemical characteristics.

Land use system significantly affected the soil physical and chemical factors which in turn influenced the occurrence of *Trichoderma* spp. Forests soils were high in C, N, Fe, OM and Mg and this favored the occurrence of this fungus. Mineral nutrition is essential for growth, sporulation and stimulation of fungal secondary metabolism (Griffin, 1994). High total N availability increased sporulation, production of antifungal anthroquinone pigments, hyphal growth rate (Fargasova, 1992), and antagonistic activity of *Trichoderma* spp. against wood rot fungus *Serpula lacrymans* (Score and Palfreyman, 1994). Soil nitrate levels were positively correlated with cellulose production (Widden and Breil, 1988) and may favor competitiveness of the bio-control agent with the pathogen. Magnesium increased growth of *T. viride* (Shukla and Mishra, 1970), and copper enhanced conidiogenesis and biomass nitrogen in other hyphomecetes (Ismail *et al*., 1991; King et al., 1982). In the ordinations between LUTs and soil properties, the first two factors accounted for 45.67% of the total variations in the data, grouping forests on one side and

cultivated fields on another. One of the factors could be soil management system with soil inputs in the cultivated fields influencing the soil properties with the second factor being tillage. Variability in land management results in varying soil characteristics as seen among the fallows. The fallows could vary in the length of time they were left to lie idle and even the types of crops planted whenever farming resumed. This variation resulted in soil differences listing a fourth factor that contributed to varying soil factors and thus determined the occurrence of the fungus. Soils under napier LUT recorded the highest frequency of isolation yet the ordinations grouped napier together with other cultivated soils indicating that there was another factor, apart from the soil properties that was unique to napier fields that attracted the fungus. This could be related to the extensive rooting system of the grass as explained above indicating plant type as yet another driver of soil microbial community structure. Sariah *et al.,* (2005) also reported that *Trichoderma* abundance and distribution was crop specific.

Soil texture was another factor that correlated with *Trichoderma* distribution. The forests with clay loam soils favored fungal occurrence rather than clay soils found the cultivated fields. The proportions of soil textural components such as clays has a major influence on water movement, oxygen availability, and the matrix for fungal growth and dispersal (Schippers *et al*., 1987).Clay minerals have been reported to reduce respiration of *T. viride* (Stotzky and Rem, 1967).

CONCLUSION

Soil type and plant type determine distribution of *Trichoderma*. The mode of exertion of the function of these two major drivers was a complex interaction between for example the fungus and soil, plants, and other soil microorganisms. In some situations it was the soil that was the key factor determining fungal occurrence and diversity while in others it was the plant type.

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